Evidence of an interaction between nifedipine and nafcillin in humans

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Aims Nafcillin (Wyeth Laboratories, Philadelphia, PA, USA) has been reported to induce the metabolism of cyclosporin and warfarin, which are known substrates of cytochrome P-450 (CYP). However, there has not been any report to date on its possible interaction with nifedipine, an index substrate of the enzyme, CYP3A4.

Methods Nine healthy normotensive subjects participated in this randomized placebo-controlled two-way crossover study examining the effects of 5 days' pretreatment of nafcillin 500 mg or placebo four times daily on the pharmacokinetics of an oral dose of nifedipine 10 mg. Plasma nifedipine concentrations were measured by gas chromatography–mass spectro.

Results The area under the plasma nifedipine concentration–time curve ($AUC_{0-\alpha}$) in nafcillin-pretreated subjects (80.9 ± 32.9 μg l^{-1} h^{-1}) was significantly decreased compared with subjects who received only nifedipine (216.4 ± 93.2 μg l^{-1} h^{-1}) ($P < 0.001$). Total plasma clearance of nifedipine (CL/F) was significantly increased with nafcillin pretreatment (138.5 ± 42.0 l h^{-1} vs 56.5 ± 32.0 l h^{-1}) ($P < 0.002$).

Conclusions The results show that nafcillin pretreatment markedly increased the clearance of nifedipine and suggest that nafcillin is a potent inducer of CYP enzyme.

Keywords: cytochrome P450, drug interaction, nafcillin, nifedipine

Introduction

Nafcillin is a semisynthetic penicillin analogue that remains in clinical use because of its activity against penicillinase-producing *Staphylococcus aureus* [1]. It is absorbed in the intestine and is primarily eliminated via hepatic metabolism [2]. Less than 30% of administered nafcillin is excreted renally, with approximately 8% eliminated in the bile [3].

In a study by Veremis *et al.* [4], it was reported that nafcillin decreased the concentration of the immunosuppressive agent, cyclosporin. The activity of CYP3A enzyme is critical to the biotransformation of cyclosporin and is the rate-limiting step in the elimination of cyclosprorine [5]. In another case study by Qureshi *et al.* [6], it was found that nafcillin enhanced warfarin elimination. The interaction did not appear to be related to warfarin's absorption and extent of protein binding but rather to apparent hepatic microsomal enzyme induction. These findings suggest that nafcillin may be an inducer of CYP activity. In this study, we have tested this hypothesis by investigating the effect of nafcillin pretreatment on the pharmacokinetic parameters of nifedipine, an index substrate of CYP3A in humans.

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Methods

Subjects

Nine male volunteers, ranging from 21 to 23 years of age and weighing from 45 to 70 kg, gave their written informed consent to participate in this study protocol, which was approved by the Ethics Committee of the University of Malaya Medical Centre. All of the subjects were considered to be healthy, as determined by a thorough physical examination, medical history and biochemical and haematological blood testing. None of the subjects was taking any other medication prior to or during the period of the study.

Study design

A randomized, placebo-controlled, two-way crossover study design was applied in this study with a washout period of 4 weeks. In each part of the study, four subjects were randomized to receive either a 500-mg dose of nafcillin (Wyeth Laboratories, Philadelphia, PA, USA) or placebo, four times daily for 5 days. Thereafter, on the day of the study, following an overnight fast, all subjects were given a single 10-mg nifedipine capsule (Adalat;

Bayer Pharma, Federal Republic of Germany) to be taken with 100 ml of water. Subjects were restrained from taking food and water for at least 3 h after nifedipine administration. All of the volunteers were kept in a study ward throughout the study period and were regularly monitored by the attending physician.

Blood samples (4 ml) were collected in vacutainers (containing sodium heparin as the anticoagulant agent) at 0 h before dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h after drug administration. An indwelling cannula was used to draw out the blood during the first 12 h of blood sampling, while the subsequent 24-h sample was taken by direct venepuncture.

The blood samples were centrifuged immediately and the plasma separated and stored at −20°C in aluminium foil-wrapped tubes or containers until the time for analysis. Vacutainers were also wrapped in aluminium foil to prevent and minimize photo-decomposition of the drug.

Drug assay

The extraction method used in this study is a modification of the method by Raemsch *et al.* [7]. A 0.5-ml sample of plasma containing nifedipine and the assay internal standard (60 μg l^{-1} diazepam; Sigma Chemical Co., St Louis, MO, USA) and 50 μl of sodium hydroxide (0.5 M), were mixed by vortexing the contents for 3 s. Toluene (2 ml) was added to this mixture and shaken for another 30 min. The organic solvent (containing nifedipine) was then separated and transferred into an amber Eppendorf tube (2 ml) and placed in a vacuum evaporator for about 50 min to allow the organic solvent to evaporate. The remaining residues were then reconstituted with 20 μl of toluene. One microlitre of this reconstituted sample was then injected into the gas chromatography–mass spectrometry for analysis.

Standard curves were prepared from plasma samples containing known concentrations of nifedipine within the range of 10–200 μg l^{-1}. The standards were prepared by mixing solutions from the stock solution of 500 μg l^{-1} nifedipine, which was prepared daily with blank plasma. The plasma recoveries with this assay ranged between 109.6% and 117.1%. The calibration curve in blank plasma was linear ($r^2 > 0.99$) from 10 to 200 μg l^{-1}. The lower limit of detection was 5 μg l^{-1} which was six times the baseline noise with 15% within-day variability. Within- and between-assay variabilities for 20, 80 and 160 μg l^{-1} were 1.9–2.8% and 3.5–7.2%, respectively.

Data analysis: pharmacokinetic and statistical

The pharmacokinetic (PK) parameters were derived from noncompartmental analysis, while parametric (ANOVA, *t*-test) and nonparametric (Wilcoxon match pair test) methods were applied to statistical analysis. PK and statistical analysis were performed using the WinNonlin Professional and SPSS software (SPSS Inc, Chicago, IL, USA). Area under the plasma concentration–time curve (AUC), plasma peak concentration (C_{max}), time to achieve C_{max} (T_{max}), total plasma clearance (CL/F), and elimination constant (λ), were calculated using the WinNonlin pharmacokinetic program. A noncompartmental approach for PK analysis provided a simple method of determining the AUC by the application of the linear trapezoidal rule. AUC was extrapolated to infinity by adding the last quantifiable plasma concentration of the drug (AUC at 24 h) divided by the λ-value to obtain the AUC to infinity ($AUC_{0-\alpha}$) values. The half-life ($t_{1/2}$) was estimated by linear regression of the last three to four data points. The mean values ± standard deviations (SD) of all parameters were compared, and 95% confidence intervals (CIs) were applied to the differences for all endpoints in this study.

Results

There was a clear difference in the plasma nifedipine concentration–time profile when subjects were pretreated with nafcillin prior to taking nifedipine (Figure 1). Plasma nifedipine levels following nafcillin pretreatment were lower than the levels detected in the same subjects when they were not pretreated with nafcillin. Nafcillin had no statistically significant effect on C_{max} and T_{max}; however, the plasma nifedipine $t_{1/2}$ was reduced 2.6-fold (Table 1). As a result, the $AUC_{0-\alpha}$ decreased by a similar amount and the CL/F was 2.4-fold greater after nafcillin pretreatment (Table 1). The CL/F values obtained in this study were similar to previous PK studies of nifedipine [8].

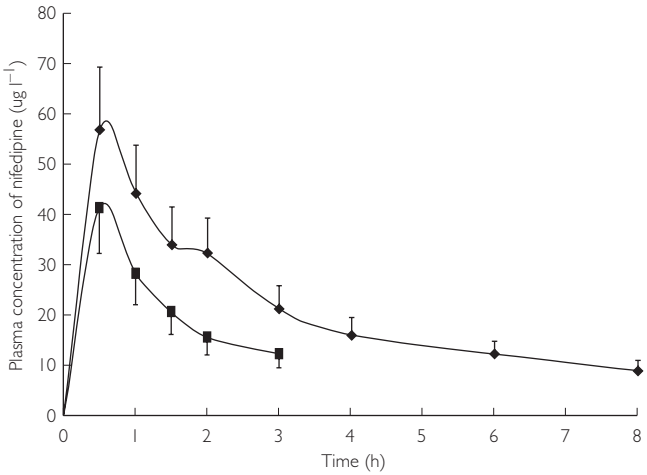

Figure 1 Plasma nifedipine concentration–time curve in nafcillin-pretreated subjects (■) and subjects who received only nifedipine (closed diamond). Results are mean ±SEM Values below the lower limit of detection (5 μg l^{-1}) have been excluded.

Table 1 Pharmacokinetic parameters of subjects given nifedipine alone and nifidepine with nafcillin pretreatment.

	C_{max}(ng ml^{-1})		T_{max} (h)		$t_{1/2}$ (h)		$AUC_{0-\infty}$ (ng ml^{-1} h^{-1})		CL/F (l h^{-1})	
Subject	Nif	*Nif + Naf*	*Nif*	*Nif + Naf*	*Nif*	*Nif + Naf*	Nif	*Nif + Naf*	*Nif*	*Nif +Naf*
1	77.0	48.5	0.5	0.5	2.3	0.8	204.7	76.5	48.9	130.8
2	59.2	65.0	1.0	0.5	3.3	0.4	195.6	70.4	51.1	142.1
3	39.7	78.0	0.5	0.5	5.0	1.1	352.1	140.8	27.9	71.0
4	35.5	59.2	1.0	0.5	4.2	1.7	177.1	133.0	56.5	75.2
5	56.1	15.1	2.0	1.5	1.8	1.8	146.5	54.3	68.3	184.3
6	144.0	36.9	0.5	0.5	2.1	0.9	365.0	77.3	27.4	129.4
7	49.9	19.2	0.5	2.0	1.0	0.7	74.5	57.3	134.2	174.6
8	38.2	41.8	0.5	0.5	3.7	1.2	247.1	61.3	40.5	163.1
9	83.7	43.5	0.5	0.5	2.8	0.9	185.2	56.8	54.0	176.2
Mean	64.8	45.2	0.8	0.8	2.9	1.1	216.4	80.9	56.5	138.5
SD	34.1	20.4	0.5	0.6	1.3	0.5	93.2	32.9	32.0	42.0
95% CI	38.6, 91.0	29.5, 61.0	0.4, 1.2	0.3, 1.2	1.9, 3.9	0.7, 1.4	144.8, 288.1	55.5, 106.2	31.9, 81.1	106.2, 170.8
P-value		NS		NS		<0.002		<0.001		<0.002

Discussion

Nafcillin has been reported to reduce plasma levels of cyclosporin, a substrate of CYP3A enzyme [4]. Taken together, our findings would suggest that nafcillin is a potent inducer of CYP3A enzyme, although it should be noted that the effect of nafcillin on plasma protein binding was not investigated in this study. While the induction of CYP3A is pregnane X receptor (PXR)-mediated [9], the relationship between nafcillin induction of nifedipine elimination and PXR is unknown and cannot be inferred from this study. It should also be noted that there have been several case reports of enhanced warfarin elimination during concomitant nafcillin administration [6, 10, 11]. Warfarin occurs as a pair of enantiomers that are differentially metabolized by CYP. Although R-warfarin is metabolized by CYP3A to 10-hydroxywarfarin, this pathway is only a minor one [12]. R-warfarin is primarily metabolized by CYP1A2 to 6- and 8-hydroxywarfarin. The other enantiomer, S-warfarin, is primarily metabolized by CYP2C9 [12]. Although potential warfarin–drug interaction could occur with any of a wide range of drugs that are metabolized by these CYP enzymes, the efficacy of warfarin is largely affected only when metabolism of S-warfarin is affected [12]. The mechanistic link between nafcillin induction of CYP3A and that of CYP2C9 is not known. These observations may suggest that nafcillin is a nonselective inducer of CYP enzymes. Clearly, further studies are required to investigate the CYP enzyme-inducing effect of nafcillin.

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